



MASSACHUSETTS
GENERAL HOSPITAL

TRAUMA CENTER



HARVARD
MEDICAL SCHOOL

Hasan Alam, MD
165 Cambridge Street, Suite 810
Boston, MA 02114
617-643-2433

May 15, 2007

Michael B. Given, Ph.D.
Program Officer, Casualty Care Management
Office of Naval Research
875 North Randolph Street, Suite 14 (Code 341)
Arlington, VA 22203-1995.

Ref: Final Report Submission for ONR Awards no: N00014-05-MP-2-0006 (Uniformed Services Univ./HJF) and N00014-06-1-0192 (Massachusetts General Hospital).
Principal Investigator: Hasan Alam, MD

Dear Dr. Given,

Attached is the final technical report for the above referenced project. This project was completed at two separate institutions, because I moved from the Uniformed Services University (USUHS) to the Massachusetts General Hospital (MGH) in 2005. The unused funds on this project were returned by the USUHS to the Office of Naval Research, and reissued as a new grant to the MGH. This is reflected on the face sheet, which contains two sets of grant/project numbers and a longer overall period of reporting.

Six copies of the completed package have been distributed as required. I am also sending you copies of the newer manuscripts that have been published since the last report. I hope that you find the report satisfactory. Please do not hesitate to contact me in case you have any questions or need any more information.

Sincerely,

Hasan B. Alam, MD, FACS
Massachusetts General Hospital/ Harvard Medical School, Boston, MA.
Director of Research. Division of Trauma, Emergency Surgery, and Surgical Critical Care

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 05/17/2007		2. REPORT TYPE Final Technical Report		3. DATES COVERED (From - To) 10/01/2002-02/28/2007	
4. TITLE AND SUBTITLE Far Forward Treatment of Hemorrhagic Shock				5a. CONTRACT NUMBER N0001405MP20006 / N000140610192	
				5b. GRANT NUMBER MDA905-03-01-0004	
				5c. PROGRAM ELEMENT NUMBER	
				5d. PROJECT NUMBER G190JA-01 / 07PR02566-01	
6. AUTHOR(S) Hasan B. Alam, MD				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Henry M. Jackson Foundation for the Advancement of Military Medicine 1401 Rockville Pike, Suite # 600, Rockville, MD 20852 Massachusetts General Hospital (The General Hospital Corp.) 50 Staniford Street, 10th floor, Suite 1001, Boston, MA 02114				8. PERFORMING ORGANIZATION REPORT NUMBER 600-090-00000-00-102456 / 203523	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Henry M. Jackson Foundation for the Advancement of Military Medicine 1401 Rockville Pike, Suite # 600, Rockville, MD 20852 Massachusetts General Hospital (The General Hospital Corp.) 50 Staniford Street, 10th floor, Suite 1001, Boston, MA 02114				10. SPONSOR/MONITOR'S ACRONYM(S) HJF / MGH	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) 600-090-00000-00-102456 / 203523	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for Public Release: Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Hemorrhagic shock is the leading cause of death in civilian and combat trauma. Even when the injured survive long enough to be transported to a medical facility, hemorrhage still remains the leading cause of preventable late death and complications. Effective hemorrhage control and better resuscitation strategies have the potential of saving lives. However, resuscitation can exacerbate cellular injury caused by hemorrhagic shock. Utilizing the funding provided by the ONR, we have clearly established that resuscitation fluids play a critical role in this injury pattern. Furthermore, we have demonstrated that these adverse effects can be avoided through simple modifications. We have also designed novel strategies for cellular protection. In parallel, advanced hemostatic battlefield dressings have been developed and validated. The goal of this research has been to improve the care of the critically injured, and a number of our findings have already been incorporated into new military doctrine (e.g. use of new hemostatic dressings, limited volume resuscitation), saving numerous lives.					
15. SUBJECT TERMS Hemorrhage, hemorrhage control, resuscitation, fluids, battlefield injuries, battlefield dressings, hypothermia, devices, pump.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Hasan B. Alam, MD
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) 617-643-2433

FINAL TECHNICAL REPORT

GRANT #: N00014-05-MP-2-006 (Uniformed Services Univ./HJF) and N00014-06-1-0192 (Massachusetts General Hospital)

PRINCIPAL INVESTIGATOR: Hasan B. Alam, MD

INSTITUTION(S): Uniformed Services University of the Health Sciences, Bethesda and Massachusetts General Hospital, Boston (PI moved to Boston in 2005).

GRANT TITLE: Far Forward Treatment of Hemorrhagic Shock

AWARD PERIOD(S): October 1, 2002- September 30, 2005 (Uniformed Services University). November 21, 2005- November, 20, 2006 (Massachusetts General Hospital).

REPORTING PERIOD: The period covered by this report is October 1, 2002-February 28, 2007.

OBJECTIVE:

The overall goal of our research was to improve the outcome of combat casualties. During this period of funding, three complimentary areas of research were pursued. First, we studied different methods of resuscitation following hemorrhagic shock. Second, we developed and tested surgical techniques and equipments to improve the care of injured soldier in the battlefield. Third, we tested various hemostatic agents to identify the best agent for use in the battlefield.

APPROACH:

Resuscitation (Area 1): Our team developed a number of small and large animal model of hemorrhagic shock. Using these clinically relevant animal models, the impact of various resuscitation strategies on cellular injury, organ function, hemodynamic profile, and immune/inflammatory response was studied. We also developed novel resuscitation fluids that did not exhibit the adverse properties of conventional fluids. These new fluids were then extensively tested in a variety of pre-clinical models.

Development and testing of new equipment (area 2): We wanted to demonstrate that development of new equipment and refinement of techniques would make life saving interventions suitable for battlefield application. Two such devices were developed and tested in appropriate animal models, including a small portable pump for induction of hypothermic metabolic arrest (following lethal uncontrolled hemorrhage) and a simple, portable device for evacuation of air and blood from the body cavities (e.g. blood from pleural space).

Hemorrhage control (area 3): We developed a lethal but potentially salvageable swine model of severe groin injury. This model was used to perform a series of pre-clinical

trials, which resulted in FDA approval of a new zeolite hemostat. This zeolite hemostat (QuikClot) and another dressing that was tested in these experiments (HemCon) have been deployed to the battlefield. We also tested various new formulations of zeolites that were developed to mitigate the exothermic reaction (seen with the original QuikClot) and make the product easier to use.

ACCOMPLISHMENTS (Throughout award period):

1. **Resuscitation:** It is now well recognized that administration of resuscitation fluids is not completely innocuous and may actually augment post-shock cellular damage. In an effort to design better resuscitation techniques, we have studied in detail the mechanisms by which resuscitation strategies affect cellular functions. Our experiments have demonstrated that resuscitation with the conventional lactated Ringer's solution (mixture of D- and L-isomers of lactate) results in an up-regulation of various markers of cellular injury, including programmed cell death (apoptosis). Apoptosis in key organs can be markedly reduced by eliminating D-lactate, and by substituting lactate with beta-hydroxybutyrate (ketone Ringer's) or sodium pyruvate (pyruvate Ringer's). Influenced by this emerging data about the adverse effects of D-lactate, one major manufacturer of resuscitation fluids (Baxter Corp) has switched to using pure L-isoform of lactate.

We have also shown that these novel Ringer's solutions exert their protective effects through modifications of key regulatory proteins (nuclear and cytoplasmic). Modifications of nuclear regulatory proteins (i.e. histones) in turn influences transcription of genes, which controls a number of downstream pathways. These mechanisms are also potential targets for therapeutic interventions. For example, our data show that administration of some pharmacological agents (HDAC inhibitors) after hemorrhage can produce histone acetylation patterns that are almost identical to the ones seen after ketone Ringer's resuscitation. This approach also results in reversal of shock-induced regulatory imbalances, and improves survival.

To test various resuscitation strategies, we first used small animal (rodents) volume controlled hemorrhage models, along with in-vitro studies using human blood exposed to resuscitation fluids. After obtaining convincing comparative data, for the final study we designed a clinically relevant large animal model of shock with a number of salient features. These included: 1) uncontrolled hemorrhage from intra-abdominal vascular injuries, 2) three phases of resuscitation simulating pre-hospital, intra-operative, and the recovery periods, 3) clinically relevant volumes and types of resuscitation fluids, 4) surgical repairs of vascular injuries, 5) clinically meaningful end points of resuscitation, and 6) comprehensive monitoring of hemodynamic parameters and organ function. The findings of this experiment were presented at two premier surgical meetings (American Association for the Surgery of Trauma, and the American College of Surgeons), and some of the findings are already in peer reviewed literature (Ayuste et al. J Trauma 2006). This study confirmed the earlier findings that resuscitation with conventional lactated Ringer's causes a significant increase in apoptotic cell death in lung and liver. Furthermore, this can be avoided if

D-lactate is eliminated from the resuscitation fluid. We also noted that, similar to rodents, resuscitation strategies influence cellular regulation in swine at the level of gene transcription through differential acetylation of histone proteins.

2. **New Equipment:** Two devices were developed and tested in appropriate animal models during this period. The first was a small portable pump developed by the Cleveland Clinic Foundation for the induction of hypothermic metabolic arrest. This was tested in a swine model of lethal uncontrolled hemorrhage and found to be very effective (Alam *J Trauma* 2006 and Casas *Artificial Organs* 2005). As this pump was very small, disposable, battery operated and very cost effective, we found it to be logistically superior to the conventional hear-lung bypass machine. A simple, portable device for evacuation of air and blood from the body cavities was developed and a patent filed (Alam 2003). This device has been licensed by Bard Inc. and will be marketed for evacuation of pleural effusions in the summer of 2007. We are currently negotiating possibilities for licensing and development of a similar device for the evacuation of pleural blood in trauma victims.
3. **Hemorrhage control:** Uncontrolled hemorrhage remains the major cause of preventable battlefield deaths. Identification of an effective battlefield dressing has been the focus of active research in our lab over the last few years resulting in the deployment of zeolite hemostat (QuikClot) by the Marine Corps. Although very effective, zeolites generate heat when exposed to blood and there has been some concern about potential for tissue injury with their use. Zeolite granules are also difficult to remove from the wounds and a better delivery system is needed to facilitate application and removal of the hemostat. In 2005, we worked in close collaboration with another group that has been funded by the ONR (Galen Stucky, PhD, Univ. Calif. Santa Barbara) to develop a new generation of zeolites that are less exothermic. Using an ion substitution method, Dr. Stucky's team replaced calcium ion in the standard QuikClot zeolite with a number of less exothermic ions to manufacture a new generation of zeolites. After initial in-vitro screening, promising versions were selected for further in-vivo testing by our team at USUHS. Using a swine model of lethal groin injury (femoral artery, vein and soft tissues) we screened five different formulations of the new zeolites, and a beaded version of the conventional zeolite packaged in a fabric bag. We also screened the new formulation of Chitosan dressing (HemCon, Hemorrhage Control Technologies, Lake Oswego, OR), and two new products: 1) Hemostatic Polymer Bandage (ARES Lab, West Sacramento, CA) and, 2) Chitosan-starch polymer fleece dressing (developed by Loma Linda University, and the Medafor Corporation, Minneapolis, MN). Based on the results of the screening, three new zeolites (Ag, Ba, and Na exchanged formulations), bagged zeolite, and HemCon were selected for a randomized pre-clinical trial where use of the bagged zeolite was associated with 90% survival (compared to 0% in control group). The maximum wound temperatures after the application of zeolites was not very different in this study (48-55°C) compared to our previously published data (51-57°C). However, in contrast to our last study (Alam et al. *J Trauma* 2004), almost no histological evidence of tissue injury was noted in the current study. Possible reasons for the lack of tissue injury may include differences in

the rate of heat generation, duration of exothermic reaction, and physical barrier between the zeolite and tissues (in the bagged zeolite group). These possibilities deserve further investigation. These data were presented at the ATACCC meeting (August 2005, St. Petersburg, FL), and the Western Trauma Association annual meeting (February 2007), and the full manuscript has recently been published in the Journal of Trauma (Ahuja 2007). In all, we have published 3 original studies and 2 review papers on this topic as listed in the manuscript section.

CONCLUSIONS:

1. Exuberant activation of circulating white blood cells through excessive fluid resuscitation can cause wide spread organ injury. This is especially true for conventional lactated Ringer's solution and artificial colloids.
2. Cellular injury in various organs can be reduced through modifications of the conventional Ringer's solutions.
3. Elimination of D-isomer of lactate from the lactated Ringer's solution is clearly beneficial. Additional cellular protection can be achieved by substituting lactate with equal amounts of a ketone body (beta-hydroxybutyrate) or sodium pyruvate.
4. Resuscitation strategies can influence key cellular regulatory mechanisms in a fluid specific fashion. These include; 1) transcriptional regulation of genes through modifications of nuclear histone proteins, and 2) post-translational modifications of proteins involved in cell survival.
5. Development of new equipment can provide logistical advantage in austere environments, and potentially make life saving interventions suitable for battlefield application.
6. Induction of profound total body hypothermia can preserve viability of key organs during repair of lethal injuries and improve survival following lethal injuries. Protective hypothermia can be induced using portable, battery operated, small pumps.
7. Rapid and effective hemorrhage control improves survival following lethal injuries.
8. Advanced hemostatic dressings are superior to standard dressings for control of lethal external hemorrhage.
9. Advanced hemostatic dressings can be refined to improve their safety profile and while maintaining the efficacy.

SIGNIFICANCE: The goal of our research is to improve the treatment of combat casualties, and a large number of our findings have already been incorporated into new military doctrine (e.g. use of advanced hemostatic dressings, limited volume resuscitation). Historic data demonstrates that the single major cause of treatable death in combat casualty is hemorrhage. Historically, 20% of the combat casualties were killed in action (KIA), with about 90% dying before reaching the field hospital. The cause of death is hemorrhage in 50% and neurologic trauma in 36% of these soldiers. The rest are from devastating multiple injuries. Even when the injured survive long enough to be transported to a medical facility, hemorrhage still remains the leading cause of

preventable late death and complications. In a review of Vietnam War data, almost 40% of soldiers that were KIA due to exsanguination had a source of hemorrhage that could have been controlled by simple hemostatic measures.

Due to the changing strategies of engagement in future military conflicts, medical support must also change in order to accommodate future needs. Today, battlefronts are more rapid and non-linear. The logistical footprint is much smaller and often there are no full fledged hospitals in close proximity to the frontlines. Conflicts in urban settings are often associated with delays in evacuation. Therefore, the treatment strategies for the injured must adjust to accommodate the changing scenarios of conflicts. Due to the recent advances combat casualty care (and protective armor), the KIA rate in the ongoing conflict in Iraq and Afghanistan is the lowest in the history of modern warfare.

PATENT INFORMATION:

US Provisional Patent Application (serial no. 60/523,321) entitled "Portable Hand Pump for Evacuation of Blood" filed on November 20, 2003.

AWARD INFORMATION:

During the period of this funding the principal investigator has received a number of awards and academic promotions:

Awards: Recognition by the US Marine Corps for contributions to Combat Casualty Care (2003). Edward E. Cornwell Award for outstanding surgical educator (2005). W. Gerald Austen, MD Scholar in Academic Surgery, Massachusetts General Hospital/Harvard Medical School (2005).

Academic/administrative promotions: Associate Professor of Surgery at USUHS (2003-present). Associate Professor of Surgery at Georgetown Univ. (2004-2005). Associate Professor of Surgery at Harvard Medical School (2005-Present). Director of Surgical Research at Washington Hospital Center, Washington, DC (2002-2005). Director of Trauma Research and Readiness Institute for Surgery at USUHS (2004-2005). Director of Research for Trauma, Emergency Surgery and Surgical Critical Care at the Massachusetts General Hospital, Boston (2005-Present).

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19. Jaskille A, Koustova E, Kirkpatrick JR, Rhee P, **Alam HB**. Hypertonic saline resuscitation attenuates pulmonary apoptosis in rats through a PI3K-Akt independent pathway. *J Surg Res* 121(2):336, 2004.
20. Alam HB, Honma K, Ayuste EC, Huang W, Koustova E, Rhee P, Chen Z. Rapid induction of profound hypothermia improves outcome in a swine model of lethal hemorrhage and complex vascular injuries. *Circulation* 110 Supp (17):III-1097, 2004.

21. Ahuja N, Koustova E, Kirkpatrick J, Rhee P, Chen H, Lin T, and Alam HB. Acetylation of cardiac histones and expression of histone regulated genes can be modulated through fluid resuscitation in a swine model of hemorrhage. *J Am Coll Surg* 201 (Supp):S24, 2005.
22. Ayuste EC, Chen H, Koustova E, Rhee P, Ahuja N, and Alam HB. Hepatic and pulmonary apoptosis following hemorrhagic shock in swine can be reduced through modifications of conventional Ringer's solutions. *J Trauma* 59:529, 2005.
23. Koustova E, Chen H, and Alam HB. Genes in hemorrhagic shock: Treatment-Time-Organ continuum. *J Trauma* 59:530, 2005.
24. Ahuja N, Ostomel TA, Stucky GA, Gonzales E, Chen Z, Rhee P, Velmahos G, de Moya M, Alam H.B. Testing of modified hemostats in a swine model of lethal groin injury. *J Trauma* 60:251, 2006.
25. Alam HB, Casas F, Chen Z, Smith WA, Reeves A, Velmahos G, de Moya M, Rhee P. Development and testing of portable pump for the induction of profound hypothermia in a swine model of lethal vascular injuries. *J Trauma* 60; 251, 2006.
26. Lin T, Koustova E, Kirkpatrick JR, Chen H, Alam HB. Histone deacetylase as a therapeutic target in hemorrhagic shock: effects of fluid resuscitation strategies. *J Surgical Research* 130(2); 281, 2006.

NATIONAL/INTERNATIONAL MEETINGS

1. February 2006- 36th annual meeting of the Western Trauma Association, Big Sky, Montana. Oral Presentation. Alam HB et al. Development and testing of portable pump for the induction of profound hypothermia in a swine model of lethal vascular injuries.
2. February 2006- 36th annual meeting of the Western Trauma Association, Big Sky, Montana. Oral Presentation. Ahuja et al. Testing of modified hemostats in a swine model of lethal groin injury.
3. February 2006- 67th annual meeting of the *Society of University Surgeons*, San Diego, CA. Oral/poster presentation. Lin T, Koustova E, Kirkpatrick JR, Chen H, Alam HB. Histone deacetylase as a therapeutic target in hemorrhagic shock: Effects of fluid resuscitation strategies.
4. October 2005- 91st *Clinical Congress of the American College of Surgeons (Surgical Forum)*, San Francisco, CA. Oral presentation. Ahuja N, Ayuste E, Koustova E, Kirkpatrick J, Rhee P, Alam HB. Acetylation of cardiac histones and expression of histone regulated genes can be modulated through fluid resuscitation in a swine model of hemorrhage.
5. September 2005- 65rd Annual meeting, *American Association for the Surgery of Trauma*, Atlanta, GA. Oral presentation. Ayuste E, Ahuja N, Koustova E, Rhee P, Alam HB. Hepatic and pulmonary apoptosis following hemorrhagic shock in swine can be reduced through modifications of conventional Ringer's solution.
6. September 2005- 65rd Annual meeting, *American Association for the Surgery of Trauma*, Atlanta, GA. Oral presentation. Koustova E, Chen H, Alam HB. Genes in hemorrhagic shock: Treatment-Time-Organ continuum.
7. February 2005- 66th annual meeting of the *Society of University Surgeons*, Nashville, Tennessee. Oral presentation. Lin T, Koustova E, Rhee P, Kirkpatrick JR, and Alam HB. Histone deacetylase as a therapeutic target in hemorrhagic shock.

8. November 2004- 38th annual meeting, *The Association for Academic Surgery*, Houston, TX. Poster presentation. Jaskille A, Koustova E, Kirkpatrick JR, Rhee P, Alam HB. Hypertonic saline resuscitation attenuates pulmonary apoptosis in rats through a PI3K-Akt independent pathway.
9. October 2004- 90th *Clinical Congress of the American College of Surgeons (Surgical Forum)*, New Orleans, LA. Oral presentation. Jaskille A, Koustova E, Rhee P, and Alam HB. Hepatic apoptosis following hemorrhagic shock in rats can be reduced through modifications of conventional Ringer's solutions.
10. September 2004- 64rd Annual meeting, *American Association for the Surgery of Trauma*, Maui, Hawaii. Oral presentation. Lin T, Koustova E, Rhee P, and Alam HB. Monocarboxylate containing resuscitation fluids activate astrocytes and microglial cells in rat brains following hemorrhagic shock.
11. June 2004- 27th *Annual Conference on Shock*, Halifax, Nova Scotia, Canada. Poster presentation. Alam HB, Chen H, Rhee P, Koustova E. Expression of Toll-like receptors following hemorrhagic shock is tissue and resuscitation specific.
12. June 2004- 27th *Annual Conference on Shock*, Halifax, Nova Scotia, Canada. Poster presentation. Lin T, Alam HB, Rhee P, Koustova E. Energy substrate-supplemented resuscitation affects brain monocarboxylate transporter levels and gliosis in rat model of hemorrhagic shock.
13. March 2004- 6th *World Congress on Trauma, Shock, Inflammation and Sepsis*, Munich, Germany. Paper presentation. Gushchin V, Alam HB, Kirkpatrick JR, Rhee P, Koustova E. Fluid induced human neutrophil activation co-relates with changes in expression of redox-sensitive genes.
14. March 2004- 6th *World Congress on Trauma, Shock, Inflammation and Sepsis*, Munich, Germany. Oral presentation. Jaskille A, Alam HB, Hancock T, Rhee P, Kirkpatrick J, Koustova E. Hepatic injury after hemorrhage and resuscitation in rats: influence of novel resuscitation fluids.
15. March 2004- 6th *World Congress on Trauma, Shock, Inflammation and Sepsis*, Munich, Germany. Oral presentation. Inocencio R, Chen H, Alam HB, Rhee P, Koustova E. Effects of resuscitation strategy on extracellular matrix: choice between destruction and protection.
16. September 2003- 63rd Annual meeting, *American Association for the Surgery of Trauma*, Minneapolis, Minnesota. Oral presentation. Jaskille A, Alam HB, Hanes W, Rhee P, Kirkpatrick J, Koustova E. Lactate induces pulmonary apoptosis by restricting phosphorylation of bad protein on serine 136, but not on serine 112, in a rat model of hemorrhagic shock.
17. September 2003- 63rd Annual meeting, *American Association for the Surgery of Trauma*, Minneapolis, Minnesota. Poster presentation. Alam HB, Jaskille A, Querol R, Chen Z, Ulloa J, Hancock T, Inocencio T, Koustova E, Burris D, Rhee P. Application of a zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in swine.
18. September 2003- 5th *International Shock Congress*, Rio de Janeiro, Brazil. Paper accepted for presentation. Hancock T, Jaskille A, Rhee P, Alam HB, Koustova E. Effect of energy substrate containing fluid resuscitation on apoptosis in rats.
19. July 2003- 6th *International Brain Research Organization, World Congress of Neuroscience*. Prague, Czech Republic. Hancock T, Alam H, Rhee P, Koustova E.

- Poster Presentation. Energy substrate supplemented resuscitation increases brain monocarboxylate transporter levels in rat model of hemorrhagic shock.
20. July 2003- *International Symposium ANA Molecular Signaling*. Vienna, Austria. Poster Presentation. Hancock T, Alam H, Rhee P, Koustova E. Energy substrate supplemented resuscitation increases brain monocarboxylate transporter levels in rat model of hemorrhagic shock.
 21. June 2003- *26th Annual Conference on Shock*. Phoenix, AZ. Poster presentation. Hanes W, Alam HB, Koustova E. A comparison of RNA isolations and chemiluminescent detection systems in membrane based gene arraying procedure.
 22. June 2003- *26th Annual Conference on Shock*. Phoenix, AZ. Poster presentation. Jaskille A, Hanes W, Alam H, Koustova E. Resuscitation with ketone Ringer's solutions activates PI3K/AKT- dependent survival pathways in rat lungs.
 23. April 2003- Annual meeting of the *Federation of American Societies for Experimental Biology (FASEB)*, San Diego, CA. Poster presentation. Inocencio R, Chen H, Rhee P, Alam HB, Koustova E. Metabolic modulations after energy substrate-supplemented resuscitation in rat model of hemorrhagic shock.
 24. February 2003- *Society of University Surgeons 64th annual meeting*, Houston, TX. Oral presentation. Koustova E, Rhee P, Inocencio R, Chen H, Hancock T, Valeri CR, Alam HB. Ketone and Pyruvate Ringer's solutions decrease pulmonary apoptosis after hemorrhagic shock and resuscitation by post-translational modification of apoptotic proteins.
 25. February 2003- *Western Trauma Association 33rd annual meeting*, Snow Bird, Utah. Oral Presentation. Jaskille A, Rhee P, Inocencio R, Hancock T, Koustova E, Seufert A, and Alam HB. Portable hand pump is effective in the treatment of hemo-pneumothorax
 26. January 2003. *Society of Critical Care Medicine 32nd Critical Care Congress*, San Antonio, TX. Poster presentation. Inocencio R, Chen H, Rhee P, Koustova E, Alam H. Extracellular matrix remodeling genes are influenced by resuscitation strategy in a rat model of hemorrhagic shock.
 27. October 2002. *88th Clinical Congress of the American College of Surgeons (Surgical Forum)*, San Francisco, CA. Oral presentation. Gushchin V, Alam HB, Kirkpatrick JR, Rhee P, Koustova E. Transcriptional profiling in leukocytes exposed to hypertonic resuscitation fluids.
 28. September 2002. *Tenth Congress of the European Shock Society*, Oslo, Norway. Poster presentation. Hancock T, Alam HB, Rhee P, Koustova E. Energy substrate containing resuscitative fluids attenuate resuscitation induced pulmonary apoptosis.
 29. September 2002- 62nd annual meeting of the *American Association for the Surgery of Trauma (AAST)*, Orlando, FL. Oral presentation. Alam HB, Stanton K, Rhee P, et al. Human polymorphonuclear cell death following exposure to hypertonic fluids in vivo: Apoptosis versus necrosis.
 30. September 2002- 62nd annual meeting of the *American Association for the Surgery of Trauma (AAST)*, Orlando, FL. Poster presentation. Alam HB, Llorente O, Uy GB, Miller D, et al. Do hemostatic agents decrease bleeding compared to standard field dressing in a swine model of lethal uncontrolled hemorrhage.

31. June 2002- 25th *Annual Conference on Shock*. Big Sky, Montana. Poster presentation. Gushchin V, Alam H, Kirkpatrick JR, Rhee P, Koustova E. Resuscitation fluids activate stress genes in human leukocytes.
32. June 2002- 25th *Annual Conference on Shock*. Big Sky, Montana. Poster presentation. Hancock T., Koustova E, Rhee P, Alam H. Effects of energy substrate containing fluid resuscitation on adenylate nucleotide contents in rats.
33. April 2002- Experimental Biology 2002. Annual meeting of the *Federation of American Societies for Experimental Biology* (FASEB). New Orleans. Poster presentation. Llorente O, Stanton K, Alam HB, Koustova E. Induction of apoptosis in human neutrophils by hypertonic and isotonic resuscitative fluids.

OTHER ACADEMIC ACTIVITIES (INVITED LECTURES- NATIONAL/INTERNATIONAL MEETINGS)

1. April 2007- *American College of Surgeons 35th Annual Spring Meeting*. Las Vegas, Nevada. Session Moderator: Current Diagnosis and Management of Deep Venous Thrombosis.
2. March 2007- *Damage Control: Reports from the War. The American Association for the Surgery of Trauma-Military Collaboration*. Bethesda, MD. Recorder, Session entitled "Damage Control: Resuscitation."
3. March 2007- *Damage Control: Reports from the War. The American Association for the Surgery of Trauma-Military Collaboration*. Bethesda, MD. Topic: Battlefield Hemorrhage Control.
4. December 2006- *Boston MedFlight Crew Meeting*, Bedford, MA. Topic: Early Resuscitation of Trauma Victims: New Developments.
5. December 2006- *Emergency Medicine Conference*. Massachusetts General Hospital, Boston, MA. Topic: Approach to Trauma Resuscitation.
6. November 2006- *CIMIT Forum*, Boston, MA. Topic: The Future of Trauma and Critical Care.
7. November 2006- *Harvard Critical Care and Trauma Symposium*, Boston, MA. Topic: Resuscitation is bad for you (the way we do it).
8. November 2006- *Harvard Critical Care and Trauma Symposium*, Boston, MA. Session Moderator: Surgery: the abdomen and the chest.
9. October 2006- *Surgical Biology Club III Program*, Chicago, IL. Topic: New Developments in Resuscitation Strategies.
10. October 2006- *American College of Surgeons 92nd Annual Congress*, Chicago, IL. Topic: Current Update on Local Hemostatics.
11. October 2006- *American College of Surgeons 92nd Annual Congress*, Chicago, IL. Topic: Induced Hypothermia for Emergency Preservation and Resuscitation (EPR) After Exsanguinating Hemorrhage.
12. October 2006- *American College of Surgeons 92nd Annual Congress*, Chicago, IL. Topic: Hypothermia for Severe Injury.
13. August 2006- Surgical Grand Rounds. Massachusetts General Hospital, Boston, MA. Topic: Suspended Animation.
14. August 2006- 21st USU Surgery Day for Trauma, Bethesda, MD. Topic: Update on hemostatic agents.

15. May 2006-MedStar Research Institute/ Washington Hospital Center, 10th Annual Research Day. Keynote Address. Topic: Reflections on an Academic Career in Surgery.
16. April 2006- Paramedic Refresher Course. Boston EMS, Boston, MA. Topic: Fluid Resuscitation.
17. February 2006-Anesthesia Grand Rounds. Massachusetts General Hospital, Boston, MA. Topic: Trauma Resuscitation: New Developments and Controversies.
18. January 2006-Cardiovascular Dysfunction in Sepsis Research Group. Massachusetts General Hospital, Boston, MA. Topic: Hemorrhagic shock and resuscitation: Effect on cell regulation.
19. December 2005-Surgical Grand Rounds. Massachusetts General Hospital, Boston, MA. Topic: Management of Abdominal Compartment Syndrome.
20. December 2005-Surgical Intensive Care Unit Core Lecture Series. Massachusetts General Hospital, Boston, MA. Topic: Therapeutic Hypothermia.
21. November 2005-10th Annual New England Regional Trauma Conference, Burlington, MA. Topic: Early Hemorrhage Control: Role of New Hemostatic Dressings.
22. November 2005-Grand Rounds. Newton-Wellesley Hospital, Newton, MA. Topic: Novel Resuscitation Strategies.
23. November 2005- Pre-hospital/Battlefield Resuscitation. *Center for Integration of Medicine and Innovative Technology* (CIMIT) Forum. Massachusetts General Hospital, Boston, MA.
24. November 2005- Trauma Research: Bench to the Bedside. *Center for Integration of Medicine and Innovative Technology* (CIMIT) Annual Briefing. Harvard Medical School, Boston, MA.
25. October 2005- Pediatric Intensive Care Unit. Massachusetts General Hospital, Boston, MA. Topic: New Developments in Resuscitation Strategies.
26. September 2005- Strategies and Solutions: The Changing Landscape of Military Ophthalmology Symposium. Schepens Eye Research Institute, Boston, MA. Topic: Hemostatic Dressings: Bench to the battlefield.
27. August 24, 2005- Division of Trauma, Emergency Surgery, and Surgical Critical Care Core Lecture Series. Massachusetts General Hospital, Boston, MA. Topic: Therapeutic hypothermia.
28. August 13-16, 2005- Advanced Technology Applications and Combat Casualty Care (ATACCC 2005). St. Petersburg Beach, FL. Topic: Immunologic consequences of resuscitation strategies.
29. August 2005- Advanced Technology Applications and Combat Casualty Care (ATACCC 2005). St. Petersburg Beach, FL. Topic: Hemostatic dressing research: an update.
30. June 23, 2005- 3rd Annual Safar Symposium, University of Pittsburgh, Pittsburgh, PA. Topic: Effect of Resuscitation Strategies on Post-Shock Inflammatory Response.
31. March 21, 2005- 2nd Fredrick W. Plugge, IV Distinguished Surgical Lecture, 25th annual USU Surgical Associates Day, Bethesda, MD. Topic: Combat Casualty Research.
32. November 2004- Visiting Professor Grand Rounds at the *Georgetown University Hospital*, Washington, DC. Topic: Advances in Resuscitation strategies.

33. November 5-6, 2004. American Heart Association Scientific sessions 2004. Resuscitative Science Symposium. New Orleans, LA. Topic: Induction of profound hypothermia to improve outcome following lethal hemorrhage.
34. October 16, 2004. The *Society of Medical Consultants to the Armed Forces*, 59th Annual meeting, Bethesda, MD. Topic: New developments in trauma care: Bench to the battlefield.
35. October 2004- *American College of Surgeons*, 90th Annual Clinical Congress. New Orleans, LA. Topic: Novel Hemostatic Agents for Control of Bleeding.
36. October 2004- Visiting Professor Grand Rounds at the *Massachusetts General Hospital*, Boston, MA. Topic: Novel Resuscitation Strategies
37. September 2004- MedStar Trauma EMS Conference "Rising to New Challenges", Washington, DC. Topic: Early hemorrhage control: Role of new hemostatic agents.
38. August 18, 2004- Advanced Technology Applications and Combat Casualty Care (*ATACCC 2004*). St. Petersburg Beach, FL.. Topic: Hemostatic dressings
39. July 12, 2004- *Committee on Tactical Combat Casualty Care* meeting. San Diego, CA. Topic: Comparative analysis of hemostatic agents for battlefield use.
40. June 2004- Conference on *Management of Battlefield Tissue Injuries*. Toronto, Canada. Topic: Use of hemostatic agents in a swine model of simulated battlefield injury.
41. April 6, 2004- Southern Society of Clinical Surgeons Annual meeting, Bethesda, MD. Topic: Combat Casualty Care Research: Bench to the Battlefield.
42. March 26, 2004- 24th Annual USU Surgical Associates Day. Bethesda, MD. Topic: Combat Casualty Care Research: Bench to the Battlefield.
43. March 24, 2004- Surgical Grand Rounds. Hospital of St. Raphael, New Haven, CT. Topic: Advances in Resuscitation.
44. January 27, 2004. Surgical Grand Rounds. Washington Hospital Center, Washington, DC. Topic: Trauma Research Program.
45. November 8, 2003. American Heart Association Scientific sessions 2003. Resuscitative Science Symposium. Orlando, FL. Topic: Resuscitative Hypothermia.
46. November 8, 2003. American Heart Association Scientific sessions 2003. Resuscitative Science Symposium. Orlando, FL. Topic: Resuscitation, current and future prospects.
47. September 16, 2003- Institute for Defense and Government Advancement, conference on Battlefield Healthcare. Arlington, VA. Topic: New Resuscitation Strategies.
48. August 25, 2003. Visiting Professor Grand Rounds at the Brooks Army Medical Center and the Institute of Surgical Research, San Antonio, TX
49. August 21, 2003. 18th Surgery for Trauma Day. Uniformed Services University, Bethesda, MD. Topic: New developments in hemorrhage control strategies for the battlefield.
50. August 18-21, 2003. Advanced Technology Applications and Combat Casualty Care (*ATACCC 2003*). St. Petersburg Beach, FL.
51. May 15, 2003. Invited Speaker at the Graduate Student Colloquium and Faculty Senate Research Day 2003. Topic: Biologic Response to Hemorrhage: Recent Advances on the Bench and the battlefield.
52. March 2003: Surgical Grand Rounds, Uniformed Services University, Bethesda, MD. Topic: Hemorrhage Control for Combat Casualties.

53. September 9-13, 2002. Advanced Technology Applications and Combat Casualty Care (*ATACCC 2002*). St. Petersburg Beach, FL.. Resuscitation with Energy substrate based solutions.
54. September 9-13, 2002. *Advanced Technology Applications and Combat Casualty Care (ATACCC 2002)*. St. Petersburg Beach, FL. Lethal injuries to extremities: Role of hemostatic agents.
55. September 9-13, 2002. *Advanced Technology Applications and Combat Casualty Care (ATACCC 2002)*. St. Petersburg Beach, FL. Anti-inflammatory and protective effects of rhIL-11 in hemorrhagic shock.